Screening of Botanical fungicides for Biocontrol of selected fungal diseases of *Aegle marmelos* and *Emblica officinalis*

SYNOPSIS

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Maharshi Dayanand Saraswati University

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(Faculty of Science, Botany)

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INTRODUCTION

Medicinal plants have been identified and used throughout human history. The two medicinal plants *Aegle marmelos* and *Emblica officinalis* play a vital role in Indian traditional and medicinal values. The area of research included in Ajmer being a holy place of worship and an optimum place for the growth of both the trees. *Aegle marmelos* considered to be ritually and medically important and so *Emblica officinalis* playing highly important role in terms of medical background.The present work deals with eradication of plant fungal diseases on these plants by natural biocontrol agents and biofungicides.

The Bael (*Aegle marmelos*) is an Indian plant, which has enormous traditional uses against various diseases. Bael (*Aegle marmelos*), family Rutaceae, is also known as Bael fruit tree, is a moderate sized , slender, aromatic tree, generally considered as sacred tree by the Hindus, as its leaves are offered to Lord Shiva during worship. Leaves, fruits, stem and roots of this tree at all stages of maturity are used as ethno medicine against various human ailments (Ganesh *et al.*, 2011).

The different parts of Bael are used for various therapeutic and curing purposes, such as for treatment of Asthma, Fractures, Healing of Wounds, Swollen Joints, High Blood Pressure, Jaundice, Diarrhoea ,Typhoid, Troubles during Pregnancy and Anaemia.

The main usage of the parts of this tree is for medicinal purposes. The unripe dried fruit of the plant is astringent, digestive, stomachic and used to cure diarrhoea and dysentery, whereas the Sweet drink prepared from the pulp of fruits produce a soothing effect on the patients who are recovering from bacillary dysentery.
The leaves are used in the treatment of opthalmia and have been claimed to be used for the treatment of various diseases like inflammation, asthma, hypoglycemia, febrifuge, hepatitis and analgesic.

Amla which is known as *Emblica officinalis* is another Indian herb which is extensively used in ayurvedic system of medicine. *Emblica officinalis*, a member of the family Euphorbiaceae is known by various vernacular names like Indian gooseberry, aonla , aamalika and amla , and is grown for the sour fruits, which have great therapeutic potential.(Sharma and Sumbali , 2012).

Indian gooseberry (*Emblica officinalis* Gaertn) commonly known as aonla is one of the important fruit crop in arid and semi arid growing regions (Jat *et al.*, 2013). Plant parts of *E. officinalis* is widely used for medicinal purposes and effective against a range of diseases like cancer, heart disease, ulcer, diabetes, liver treatment, anaemia and various other diseases.
Its mineral and vitamin contents include calcium, iron, phosphorus, carotene, thiamine, riboflavin, niacin,vitamin C (one fruit contains as much as 24 oranges) and tannins (Shaista *et al*., 2010).

In plant pathology, the term biocontrol applies to the use of microbial antagonists to suppress diseases (Heydari and Pessarakli , 2010). Plant diseases are among the main constraints affecting the production and productivity of crops both in terms of quality and quantity. Use of chemicals continues to be the major tactic to mitigate the menace of crop diseases (Jan *et al*., 2013). Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest (Paola *et al*., 2011**)**

Due to enormous medical importance of the plants and trees, it becomes primarily important to protect them from microbial organisms and other harmful diseases. Plant diseases need to be controlled to maintain the quality and abundance of food, feed and fibre produced by growers around the world (Pal and Gardener, 2006). Biological control of disease employs natural enemies of fungal pathogens to eradicate or control their population.

Biological control is the reduction of inoculums density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host or antagonist, or by mass introduction of one or more antagonists (Vishwakarma,2009).

Organic amendments are not only safe to use but also have the capacity to improve soil structure and fertility. Thus, control strategies are now directed towards the use of natural products (Sharma and Trivedi, 2002).

Importance of Proposed Investigation

Indian Medicinal plants are considered a vast source of several pharmacologically active principles and compounds, which are commonly used in home remedies against multiple ailments (Ganesh *et al.*, 2011).

Fungicides are widely used in conventional agriculture to control plant diseases. Prolonged usage often poses health problems as modern society is becoming more health-conscious. There is, however, a growing concern globally about the continuous use of synthetic chemicals on food crops because of their potential effects on human health and the environment (Ghassan *et al*., 2012).

Synthetic fungicides possess harmful negative aspects regarding various factors:

- Fungicide residues which make their way into surface and ground waters, cause adverse effects to the structure and functioning of aquatic ecosystems.
- Fungi can cause serious damage in agriculture, resulting in critical losses of yield, quality and profit. Fungal plant pathogens cause serious damages to agricultural plants every year.
- The regular use of fungicides potentially poses a risk to the environment, particularly if residues persist in the soil or migrate off-site and enter waterways.
- They potentially pose a risk to the long-term fertility of the soil.

The recurrent and indiscriminate use of fungicides have posed a serious threat to human health and to the existing human ecogeographical conditions as some of them have already been proved to be either mutagenic, carcinogenic or tetratogenic (Babu *et al*., 2008) .

In this step Biological control of plant diseases which includes fungal pathogens has been regarded as a major alternate method to substitute the harmful chemical artificial fungicides.

Major important factors regarding use of the Biocontrol method over artificial chemical fungicides are:

- They affect only the target causal organisms and related ones on the plant, where as the artificial methods affect not only the plant parts but soil, environment as well.
- Biofungicides are ecofriendly
- They are often effective in very small quantities and moreover decompose often quickly, thereby resulting in lower exposures and largely resolving the pollution problems caused by chemical fungicides.

Therefore, on the above datas, the importance of proposed investigation can be summarized as below:

1) Both the trees *Aegle marmelos* and *Emblica officinalis* are considered to be highly medically important curing many diseases.

2) Bael is considered to be spiritually important as its leaves are offered to Lord Shiva for worship.

3) Amla is highly potent form of vitamin C and is considered to be highly valuable being economical or commercial. The tannins of amla like emblicanin-A (37%), emblicanin-B (33%), punigluconin and pedunculagin are reported to provide protection against oxygen radical included haemolysis of rat peripheral blood erythrocytes (Swetha *et al*., 2014)

4) Bael fruit, also known as wood apple, contains beta-carotene, vitamins, thiamine, riboflavin and vitamin C. Bael is reported to contain a number of cumarins , proteins, carbohydrates, phenols, alkaloids , sterols and essential oils (Rashidi and Deokule , 2012**)**.

5) The fruit is known to possess significant therapeutic and medicinal value and is widely used in Homeopathy and Ayurveda. Bael is the most effective herbal remedy for diarrhoea and dysentery. Ayurveda describes Amla also as one of the best herbs for Diabetes, bleeding disorders, strength and stamina promote (Sampat *et al*., 2012).

6) As seen, Biocontrol methods on plants and trees are Ecofriendly causing lesser harm and increasing the yields both quality and quantity wise.

7) On the industrial side, attractions include cheaper discovery and registration of biocontrol agents relative to chemical fungicides, with a consequently shorter lead time.

8) Biocontrol agents have a promising future, and may offer a potential solution to the dilemma from both the environmentalists as well as industrialists.

OBJECTIVES

Fungal plant pathogens are among the most important factors that cause serious losses to agricultural products every year. Biological control of plant diseases including fungal pathogens has been considered a viable alternative method to chemical control. In plant pathology, the term biocontrol applies to the use of microbial antagonists to suppress diseases. (Heydari and Pessarakli , 2010)

Practices used in harvesting, handling, storage, production and distribution make medicinal plants subject to contamination by different fungi, which may be responsible for spoilage and production of mycotoxins (Rashidi and Deokule, 2012).

1. The main objective of the proposed investigation is to prepare biofungicides *in vitro*,made by various plant extracts and compare the same with artificial chemical fungicides by treating the fungal plant pathogen identified from the plants *in vitro*.

2. The aim is to protect the plants from harmful chemical based fungicide and use the better effective, eco friendly bio fungicide targeting the fungal plant pathogens only and yielding better and healthier results of plants.

3. To isolate and identify fungal diseases of Class Deuteromycetes in *Aegle marmelos* and *Emblica officinalis* from Ajmer and nearby localities.

4. Herbs and shrubs (*Datura stramonium,Catharanthus roseus,Nerium indicum,Calotropis procera,Ocimum sanctum*) will be collected from various localities of Ajmer, and used as partial extracts for antifungal screening test against harmful plant pathogens.

5. Comparative study and measurement of zone of inhibition of synthetic fungicides and biofungicides in plant cultures.

6. Their usage is presumed to reduce the global consumption of chemical fungicides, with little resistance problems in target organisms.

REVIEW OF LITERATURE

Some plant contains components that are toxic to pathogens. When extracted from the plant and applied on infested crops, these components can be called botanical fungicides or botanicals.

Work already done and published reports are summarized below :

Omenka *et al*. (2000) studied antimicrobial potency of grape fruit seed extracts on five selected pathogens

Nidhi *et al*. (2002) efforted on the biocontrol of root knot nematode *Meloidogyne incognita* and wilt fungus, *Fusarium oxysporum* infecting a common crop *Cuminum cyminum* .Nematicidal and Fungicidal properties were screened using plant extracts.

Pal *et al.* (2006) studied biological control of plant pathogens on the basis of mechanisms and type of interactions involved in biological control.

Babu *et al.* (2008) isolated pathogenic fungus from infected plant parts and identified based on morphological and cultural characters as *Fusarium solani f.* sp. *melongenae*. The *in vitro* efficacy of different plant were tested to control brinjal wilt pathogen.

Vishwakarma (2009) studied biocontrol of plant diseases in vegetables. The research aims to eradicate various diseases on vegetables which are important source of food consisting of vitamins, dietary and minerals.Vegetable production would increase both qualitatively and qualitatively by biocontrol of plant diseases.

Asghar *et al.* (2010) reviewed the published research works on biological control of fungal plant diseases during past 50 years. Biological control of plant diseases including fungal pathogens has been considered a viable alternative method to chemical control and applies to the use of microbial antagonists to suppress diseases.

Shaista *et al.* (2010) conducted an investigation in retailer market of three different localities viz., Hyderabad, Hala and Tando Allah Yar to examine the samples of Amla for the presence of fungal species.The aim of research was to study the fruit borne mycoflora of Amla from different localities.

Ganesh *et al.* (2011) compiled the medicinal values of *Aegle marmelos* through various research activity and approached various scientific and innovative tools.Various therapeutic purposes and phytoconstituents of the plant were revealed during the investigation.

Paola *et al.* (2011) evaluated the antifungal activity of extracts of 10 plant species used in traditional Uruguayan medicine against the phytopathogenic fungus *Alternaria* spp. The plants were selected on the basis of their reported ethnobotanical uses. Three solvents were assayed on different tissues of the plants and among the 29 evaluated extracts, 31% of the extracts inhibited growth, similar to the effects of a chemical fungicide. these extracts exhibited amazing fungicidal properties that support their traditional use as antiseptics.

Archana *et al.* (2012) evaluated the antimicrobial and antioxidant activity of fungal endophytes inhabiting *Emblica officinalis* , keeping in view the medicinal importance of the selected host plant in Indian traditional practices.

Sampath *et al*. (2012) studied various medical importance of *Emblica officinalis*. The importance of the plant covered all areas including Ayurveda and traditional and home remedies also.The research also focused on the chemical constituents and cultivation methods by which the yields of the plant can be improved.

Ghassan *et al*. (2012) investigated the effect of plant extracts as alternative synthetic fungicides in controlling mycelium growth of *Penicillium digitatum* , a pathogen for post- harvest diseases of citrus fruits. The plant extracts reported to be effective on the fungi *Penicillium digitatum* included garlic, *Withania somnifera, Acacia seyal* and *mustard horseradish.*

Rashidi *et al.* (2012) investigated the quantitative estimation of alkaloids and glycosides in association with isolated fungi . Total 20 and 15 fungal species were isolated from fresh and market root samples of drug *Aegle marmelos* .

Rohini *et al.* (2012) observed a pre harvest fruit rot in amla trees during a routine survey in Jammu . On diseased amla fruits, isolation was done and based on the fungal characters, the isolated pre harvest pathogen was identified as *Penicillium funiculosum Thom.*

Jan *et al.* (2013) worked on commercializing the biocontrol agents prepared in the laboratory and evaluating their management on the plant pathogens of various plants. The basic aim of the study is to explore the use of bio control agents for the beneficial use of living organisms and environment.

Jat *et al*. (2013) experimented on *Emblica officinalis* to see the effect of different bioagents alone and alongwith effective fungicide against aonla rust.The study was conducted in curing the plant from *Ravenalia emblicae* var. *fructicolae* Syd. and management.

Swetha *et al*. (2014) emphasized the mechanisms behind the pharmacological activities based on the recent research reports of *Emblica officinalis* and discussed the nutritional value, biochemical constituents, traditional uses, medicinal value of amla and its use as a household remedy. The work also summarizes the research done on amla for the past 5 years and also specifies the aspects that warrant future research establishing its activity and use in several diseases.

Research gaps identified in the proposed field of investigation

By Investigation, regarding the two medicinal plants taken under consideration, considerable work has been done on the antifungal and antimicrobial effects, but very less literature is available on the comparative account of natural biofungicides with synthetic chemical fungicides.

Literature has been seen regarding antimicrobial activity of endophytic fungi but not on the control measures and beneficiary methods to eradicate the fungal flora from plants. In addition, biopesticides and bioinsecticides have been considered under research and taken under commercial sectors but biofungicides has not been considered till yet.

Medicinal plants have been taken as antifungal agents in most of the research, but fungal isolation and biocontrol measures on medicinal plants has been cited very few.

As seen in most of the reviews , researches has been done considering a single fungi inhibiting a plant or a particular infected part of a plant ,but doing research on a braoder platform of isolating fungi from two medicinal plants and thereafter implementing biocontrol fungicides and comparison of the same with synthetic fungicides has not been done yet.

Therefore ,it is a matter of great importance to mould a effective research on medicinal plants *Aegle marmelos* and *Emblica officinalis* and taking a wide range of plant extracts for antifungal activity and providing a better eco friendly , economical biofungicide and thus helping to fill the Research gap.

Major Hypothesis

Living organisms (Human beings and animals) are dependent upon Plants for basic necessities including food, respiration etc.Therefore , it is an important task to protect our plants from Harmful chemical fungicides which are destroying the natural utilities both qualitatively and quantitatively. Biofungicides play a major role against the chemical fungicides being totally eco friendly and economically important. Biofungicides not only provide a healthy and nutritive zone to higher plants, but also increase the yield, quality and quantity of plants from the farmers and growers point of view, thus giving vast opportunity and source of employment to the growers. The commercial use of biofungicides thus can play a remarkable role at a global level. Plants due to regular use of synthetic harmful fungicides, have become resistant to diseased plant pathogens. Due to this, there is a severe large scale loss to plants relating to production and quality of yields. So, this is a global demand of research for biocontrol methods and biofungicides.

Research Methodology

The identification and isolation of fungal flora along with the biocontrol measures will be conducted in S.P.C. Government College, Ajmer which has a well managed Botany department including well equipped microbiology laboratory and all necessary instruments for tests.

1) Collection of Infected diseased plant parts of *Aegle marmelos* and *Emblica officinalis* will be done bimonthly in sterilized polybags from different localities of Ajmer and nearby areas. Each sample/plant part collected will be labelled and noted with date of collection along with the locality.

2) Isolation of fungi belonging to class Deuteromycetes will be done using Potato dextrose agar (Ricker and Ricker, 1936) , Czapex Dox agar (Thom and Raper , 1945) and moist blotting paper in sterilized petriplates supplemented with 100 µg/mL of streptomycin and incubated at 25 ± 2℃ for 15 days in the dark and observed daily for growth.

3) The fungal isolates will be transferred to sterilized plates for purification and identification which will be carried on the basis of portion of colony for spores and characterization of hyphae.{(Barnett (1960), Ellis (1971)}

4) Pure broth culture will be prepared under aseptic conditions.

5) 1 ml. broth suspension of fungal inoculums will be poured in the Potato Dextrose agar and Czapex Dox agar plates.

6) Antifungal sensitivity test will be screened by agar well diffusion method (Omenka and Osuoha, 2000), Dilution methods and agar disk diffusion method.

7) Herbs and shrubs will be collected for antifungal sensitivity test from various regions of Ajmer and nearby areas, which are as follows:
   - *Datura stramonium*
   - *Catharanthus roseus*
   - *Ocimum sanctum*
   - *Calotropis procera*
   - *Nerium indicum*

8) Plant extracts (partial) will be prepared aseptically in the laboratory by dissolving in different types of solvents.

   - Aqueous extract
   - Other solvents like methanol, ethanol will also be used.

   Sterilized plant parts will be kept in organic solvents such as methanol, ethanol and aqueous. Then they will be ground well with the help of Mortar and Pestle. The plant materials will be subjected to centrifugation, for 10-15 min (at 10000 rpm) again it will be filtered through filter paper. The supernatant will be collected and made to known volume, by adding sterile methanol, ethanol ,aqueous and stored for further antimicrobial screening purpose.

9) The inoculated plates will be incubated and zone of inhibition will be measured to the nearest millimeter (mm).

10) Measuring the growth of inhibition, comparative study will be made of the plant extracts and synthetic fungicides.

11) Phytochemical study will also be done.

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Signature of the Candidate with date

Outline Approved.

(Dr. Mamta Goyal)
Name and Signature of supervisor with date & seal

MAHARSHI DAYANAND SARASWATI UNIVERSITY, AJMER

Outline Of Proposed Research Work

1. Name of Scholar (In English) **SHRUTI OJHA**

(In Hindi) **Jqfr vks>k**

2. Title of the Research Work : (In English) **Screening of Botanical fungicides for Biocontrol of selected fungal diseases of *Aegle marmelos* and *Emblica officinalis*.**

(In Hindi) *bZxy ekjesyksl* o *,sEcfydk vkfQlhusfyl* ds dod jksxks ds tSo fu;a=a.k gsrq okuLifrd dod ukf'k;ksa dk ifj{k.k

3. Location

a. Institution/Department where the work is to be done : **S.P.C. Government College, Ajmer.**

b. Geographical area of Investigation, if any: **Ajmer and near by localities**

GOVERNMENT COLLEGE, AJMER

DEPARTMENT OF BOTANY

CERTIFICATE

This is to certify that Miss **SHRUTI OJHA** daughter of Late Mr. **KAILASH NATH OJHA** has Qualified the Course Work of Paper-II & III for Registration for the Ph.D. programme in the Department of BOTANY of the GOVERNMENT COLLEGE, AJMER.

Supervisor

Head of the Department